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PROFILING AND STUDY OF INTERFACIAL TENSION LADEN WITH CRUDE LIPID EXTRACT PLANT BASED AS SURFACTANT FOR FOOD APPLICATION

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ABSTRACT

Crude lipid extract plant based namely SPLIP and PULIP are being introduced in this research as a potential surfactant with phospholipid and glycolipid components which playing an important role at the oil/water interface. Since the interaction between these components give a significant impact on the interfaces, the aim of this research is to investigated whether these components in crude plant extract can also interact at oil/water interface compared to commercially available surfactant namely LEC. This work has been carried out with interfacial tension using PAT1. Prior to the interfacial tension analysis, profiling of the crude lipid extract was done using TLC. Finding obtained from TLC indicated that both crude lipid extract spotted phospholipid and glycolipid components. For interfacial results, the interaction between phospholipid and glycolipid in both SPLIP and PULIP give impact at the interfaces; being more surface active results in lower interfacial tension value.

Keywords: SPLIP, PULIP, phospholipid, glycolipid, interfacial tension

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1. INTRODUCTION

1.1. Surfactant

Surfactants are available from natural and synthetic sources and have been used in many applications such as in detergents as the foaming agent, as well as in food and pharmaceutical industry applications. In general, natural surfactants can be defined as surfactants obtained from natural sources via extraction, distillation or precipitation as they don't require further treatment or chemical synthesis. Natural sources may include plants, yeast, bacteria or animals [1].

Surfactant will be adsorbed at the interface and will subsequently alter the structural interfacial area, by displacing the water and oil molecules of the original interface. They do so by orienting themselves in such a manner that their headgroups point into water (hydrophilic) while their hydrophobic which composed of fatty acids tails point into the oil, in oil-in-water (O/W) emulsion and vice versa. A study conducted by [2] indicated that different types of monolayer formed at the oil-in-water interfaces when different types of headgroups were used as the surfactant. It was reported that the emulsion stabilised with phosphatidylcholine (PC) showed resistance to coalescence, compared to emulsions stabilised with phosphatidylethanolamine (PE) and phosphatidylserine (PS) as a more compact monolayer was formed at their interfaces. It was presumed that this could be as a result of the presence of more droplets of oil at the interfaces when PE or PS was used to stabilise the emulsion. Hence, it creates a higher possibility of oil and water interaction at the interfaces which would result in higher interfacial tension and thus more chances to coalesce.

Lipids can be good surfactants as they contain both hydrophilic and hydrophobic segments. In fact, lipids with amphiphilic characters can be claimed to be natural surfactants. Phospholipid shows amphiphilic characters as it contains both hydrophobic and hydrophilic parts; the phosphate polar headgroup is the hydrophilic part whereas the non-polar tail group, which is composed of fatty acids, is the hydrophobic. The length of both the hydrocarbon chain and the types of fatty acid also varies. These unique characteristics influence phospholipid solubility in water. Apart from phospholipid, glycolipid is also components in lipid. The main function of this glycolipid is to enhance membrane stability [3] (Chapman et al., 1983). Glycolipids are basically a glycerol moiety link to a galactose group which is a polar headgroup[4] and a

hydrocarbon chain which is a non-polar group. Glycolipid mainly consists of monodigalactodiacylglycerol (MGDG) and digalactodiacylglycerol (DGDG).

In food industries, food grade surfactants have been used; however, natural sources for food grade surfactants still need to be established further. Recently, the extraction of lipids from plants has gained much attention as they often hold dense nutrients that can be used to promote health [5]. In this study crude lipid extract from plant based were used as natural lipid surfactant due to its amphiphilic components namely phospholipid and glycolipid; which are hypothesized those crude lipid extract plant based can also be used as a surfactant. Profiling of the crude lipid extract was carried out and their interfacial tension was investigated using pendant drop technique.

2. RESULTS AND DISCUSSION

2.1 Profiling of crude lipid extract plant based

The lipid fraction extracted from the spinach chloroplast were analysed using TLC, the chromatogram for which is shown in Figure 1.0. Sunflower lecithin (SFL) and commercial MGDG and DGDG were used as a reference. Spots were visible on the TLC plate from the reference materials, these will be used as a comparison for the distances travelled by the samples. Commercial SFL (99% purity based on the manufacturer's statement) and MGDG and DGDG mixtures in chloroform solutions (98% purity based on manufacturer's normal stated purity) were spotted along with the samples onto a silica gel – they were visualised after development using iodine vaporisation. As a result, dark spots from the iodine will be observed on the TLC plate.

It can be seen that for the SFL, there were three groups of phospholipids: phosphatidylinositol (PI), phosphatidylcholine (PC) and phosphatidylserine (PS). As PC is the major component in the SFL, the PC dark spot observed on the TLC plate was dominant when compared to the other phospholipid fractions. The two major compositions of SFL are PC (41% w/w) and PI (23% w/w), while others such as phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidic acid (PA) are also present – represented by small fractions [6]. For the MGDG and DGDG mixture solutions, two spotted areas were clearly observed. As MGDG is less polar than DGDG, the interaction with the silica was weak, and therefore would elute longer

which resulted in a higher spot being observed on the plate. The opposite was true for DGDG, as DGDG is more polar than MGDG, thus DGDG had a strong interaction with the silica; as a consequence, the DGDG component could be dispelled from the silica binding place even faster. This resulted in the identification of spotting that was observed on the lower part on the TLC plate.

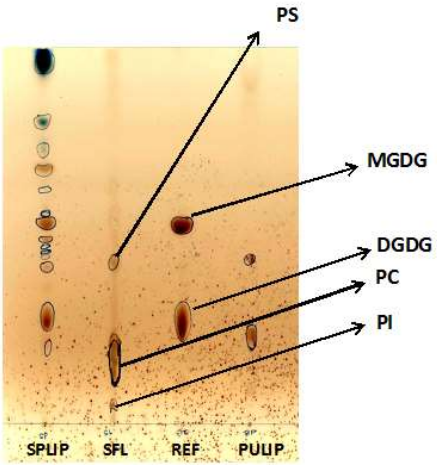

Fig.1. Thin Layer Chromatography results. Only standard labelled. SPLIP is crude lipid extract from spinach chloroplast, PULIP is crude lipid from pumpkin seed, SFL is sunflower lecithin, REF is a mixture of MGDG and DGDG standard

The spinach chloroplast (SPLIP) contained significant amounts of MGDG and DGDG as well as other components which were spotted on the TLC plate. Based on the literature, the top spotted was triacylglycerol with the second spotted being assumed to be chlorophyll.

Since the lipid fraction from the spinach chloroplasts contained a significant amount of spotted areas, detected by the TLC analysis, this solvent system is presumably the best choice for profiling purposes. Interestingly, the choice of solvent system is very important as it influences the ability of the molecules to bind with the surface of the silica gel; thus, the polarity of the solvent or a mixture of solvent will influence the way in which it will move or pass via the capillary action on the TLC plate [7]. For example, if highly polar solvents are used, the polar molecule in the solvent will interact with the polar molecules in the sample, hence there is less interaction with the binding site on the TLC plate, as such no spotting will be observed.

2.2 Interfacial behaviour of crude lipid extract

This part will investigate the interfacial tension of each of the systems in order to gain further information on the properties of the oil/water interface laden with crude lipid extract.

In order to gain information regarding the behaviour of the interfacial layers formed, the individual adsorption of each system was measured, including the purified sunflower oil/water interface, as shown in Figure 2. Purified sunflower oil/water was added as a reference, and the interfacial tension was constant at 29 ± 1 mN/m, with time. As can be seen, as soon as a drop was formed, the interfacial tension began decreasing over time, thus indicating that the surfactants begin to adsorb at the oil/water interface. However, after around 1900 seconds, a fairly constant value of interfacial tension was achieved, suggesting that the interface had become saturated with the surfactants molecules; this occurred for all of the systems and supports [8] research which noted that no further reduction of interfacial tension occurs after an interface is saturated with the surface active molecules.

All of the systems were compared, as shown in Table 1, and the interfacial tension of the LEC stabilised emulsion was the lowest, this could be because of the efficiency interaction of the hydrophobic part of lecithin with oil. The decrease in interfacial tension was proportional to the number of hydrophilic and hydrophobic groups penetrating the interface [9]; thus, indicative that the majority of lecithin was adsorbed onto the interface. Researcher [10] noted that the diffusion mechanism predominantly controlled the absorption of phospholipid onto the interface.

The presence of DGDG in the LECDG stabilised emulsion, shows slightly higher values of interfacial tension, after 2000 seconds, when compared against the LEC stabilised emulsion, the results were 17.5 ± 0.3 mN/m and 16.2 ± 0.7 mN/m, respectively; thus, the LEC stabilised emulsion system was more surface active than the LECDG system. In fact, it is suggested that the combination of high hydrophobicity and high solubility values resulted in good emulsifying surface activity [11]. However, in contrast, the results obtained by [12] reported that DGDG is more surface active than lecithin; specifically, a lower interfacial tension value was observed when the ratio of DGDG: lecithin was 1:3, compared to DGDG: lecithin at a ratio of 0:4 in the system. The concentration used in this study appears to have been much lower; this could possibly be as a result of a low concentration of DGDG being used meaning

that the interface was not saturated with DGDG and was therefore not sufficient enough to lower the interfacial tension. As such, it could be hypothesised that the adsorption was diffusion limited.

Table 1. The interfacial tension measured before and after adsorption by PAT1, at a concentration of 0.05% for all systems, the measurements were recorded at 37°C

Emulsion systems	Before (Time = 0 s)	After (Time = 2000 s)
LEC	19.8 ± 0.4 mN/m	16.2 ± 0.7 mN/m
LECDG	23.8 ± 0.2 mN/m	17.5 ± 0.3 mN/m
SPLIP	28.4 ± 0.7 mN/m	22.2 ± 0.6 mN/m
PULIP	26.8 ± 0.6 mN/m	21.5 ± 0.3 mN/m

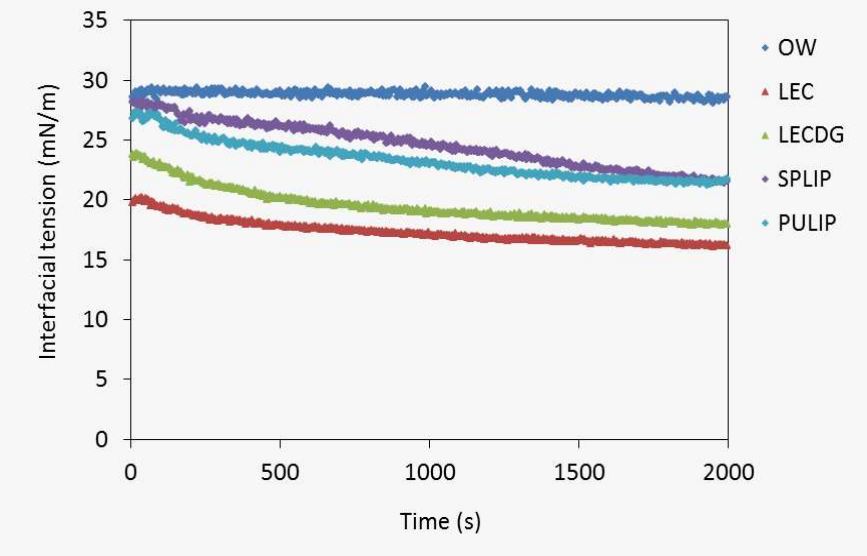

Fig.2. Interfacial tension for four types of surfactant, measured at the concentration of 0.05%, at 37°C

Oil is more viscous than water, as such the surfactant with higher molecular weight will diffuse slower in oil than in water, and will consequently adsorb slower at the interface. Due to a higher molecular weight of DGDG (937 g/mol), when compared to lecithin (815 g/mol), the surfactants possessing lower molecular weights will reach the plateau faster. Consequently, lower molecular weights aid diffusion in the bulk phases and thereby their

absorption at the interface; hence, it worth stressing that the mobility of the surfactant is dependent on the molecular weight of the surfactant itself as a higher molecular weight will result in less mobility for a surfactant.

Both the SPLIP and PULIP stabilised emulsions appeared to show similar interfacial tension values, but these values were relatively higher than those observed in the LECDG stabilised emulsion. It is possible to explain that DGDG is adsorbed at theinterface, which possesses a higher molecular weight (less mobile); thus, resulting in a slower adsorption process as well as higher interfacial tension. These findings are in agreement with the work of [13] who noted that higher interfacial tension was attained when lecithin mobility in the water-hexadecane system was lower.

To summarise, an analysis of the interfacial behaviour of four different surfactants at the oil/water interface has been presented to provide a better understanding of their physicochemical properties. The interfacial tension and data suggest that the presence of DGDG impacted on the adsorbed layer. Furthermore, there was a correlation between them being more surface active and the attainment of the lowest interfacial tension.

2. EXPERIMENTAL

1.1 Extraction from spinach leaf

The stem of the spinach leaves were cut off and weighed before being mixed with 0.3 M (M = molar) of the sucrose solution, at a ratio of 1:3 w/w, they were blended using a household blender for 30 seconds. Sucrose was used as osmotic agents, of which are commonly used in the isolation work of chloroplasts using buffer solutions. Once blended, the slurry was filtered using cheese cloth – the remaining supernatant was then poured into a 50 mL centrifuge tube and centrifuged at 3,500 g, 5°C, for 15 minutes. Finally, the supernatant was discarded and the pellet which remained was collected for further analysis. The method used for lipid extraction was adapted from Bligh and Dyer [14]. The fresh chloroplast pellet was added to a mixture of chloroform/methanol at a ratio of 2:1, it was then vortexed for 1 minute before 0.3 mL of a saline solution (0.9% NaCl) was added. NaCl was added to facilitate the partitioning of the lipids into the organic phase [15]. This mixture was then vortexed and left for 3 minutes before being centrifuged at 3,500 g, 5°C, for 15 minutes. The bottom layer, which contained

the lipids, was transferred into a pre-weigh bottle and subsequently left in a nitrogen dryer until it was dry. After which, the lipids were weighed.

1.2 Extraction from pumpkin seed

Edible pumpkins were bought in October. They were cut using a sharp knife, the seeds were collected from the gourd and then washed with tap water before drying using a dehydrator at 37°C for 3 days. Then, the dried seeds were ground using a coffee grinder.

1.3 Bulk vesicles preparation

The method referred to as hydration of a lipid cake was used to prepare the lipid vesicles because it is easy, simple and widely used [16][17]. Hydration is a process accomplished by adding an aqueous medium to the container of a dry lipid, while agitating it. For the purpose of this research, a bath sonicator was used to dissolve the dry lipid cake completely; this was followed by vigorous shaking for a recommended timeframe of an hour. Distilled water was used as the medium for hydration because the final dispersion of the hydrated vesicles would then be subsequently used as the continuous phase of the oil-in-water emulsions. The dispersion was then disrupted using a probe sonicator (Soniprep 150 Plus) at 14%, with a 230V amplitude. The disruption was carried out repeatedly until the vesicles reached a size range of between 150 and 230 nm. The solutions obtained were then centrifuged at 20,000 g for 20 minutes to remove any titanium deposits, further filtrated was performed using a 0.4 μm syringe filter. The size of the vesicles were verified based on the particle size measurement method (Delsa-nano, Beckman Coulter, UK).

Within this research, four different surfactant systems, namely lecithin, lecithin mixed with DGDG (LECDG), crude lipid extract from spinach chloroplast (SPLIP) and crude lipid extract from pumpkin seed (PULIP) were used.

1.4 Profiling of the plants extracts mixture content

1.4.1 Thin layer chromatography (TLC)

As part of the TLC, 1 mg of lipid extract was dissolved in 1 mL of chloroform. A volume of 20 μL of the extracts were spotted along with commercial sunflower lecithin (99% purity based on the manufacturer's statement, Thew Arnott, Surrey), as well as MGDG and DGDG

mixtures in the chloroform solutions (98% purity based on the manufacturer's normal stated purity), all of which were used as references on a silica gel 60 $F25_4$ HPTLC plate that was 0.2 mm in thickness. The plate was developed in a solvent system of: diisobutyl ketone, acetic acid and water (40:20:3.7), and visualised after development with iodine vaporisation. Iodine vaporisation has been used as a detection method for lipophilic substances such as indoles, amino acids, sterols and lipids [18]. Interestingly, most organic compounds will form a dark-coloured complex with iodine.

1.5 Interfacial tension

The interfacial tension of the tested samples were investigated using a Drop Shape Tensiometer (DST) (PAT1, Sinterface, Berlin, Germany) that was equipped with a single capillary. The data obtained for both interfacial tension will help to provide understanding of the behaviour of the surfactants used.

A drop was formed in a cubic glass cuvette containing purified sunflower oil. The combination of interfacial tension and the gravity, which depends on the density of the two fluids, provides shape for the drop [19]. Under gravity, the drop tends to elongate while the interfaces place a force which tends to minimise the total energy of the system to produce spherical drops. When an image of a drop was captured, the coordinates of the drop were measured using axisymmetric drop shape analysis (ADSA) software – these measurements were then compared to the profiles determined by the Gauss-Laplace equation, as shown below in Equation 1. The droplet shape and area were continuously monitored using a video-camera that was connected to a computer which had calculation software. The interfacial tension of the samples was recorded over 40 minute periods, at temperatures of 37°C.

$$\left(\frac{1}{\mathrm{R}_1}+\frac{1}{R_2}\right)=\Delta P_0+\Delta\rho\mathrm{gz} \qquad (1)$$

With regards to Equation 1: Y is the interfacial tension, R_1 and R_2 are the two principle radii of the curvature, ΔP_0 is the references pressure at an arbitrary reference plane, $\Delta\rho$ is the density difference between the fluids in contact, g is the gravity constant, and z is the vertical

height above the reference plane.

3. CONCLUSIONS

Result from TLC indicates that SPLIP and PULIP contained both phospholipid namely PC and PS and glycolipid contained DGDG were spot as compared to the standard material. Since these two components significantly affect the surfactant properties and activities, thus further investigation have been carried out. Finding via interfacial tension indicated that both interaction between the phospholipid and glycolipid practically impact on the adsorbed layer at the oil/water interface which results in different interfacial tension value. In conclusion, this research demonstrated that crude extract plant based are potentially can be used as surfactant for food application.

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